PROJECT NUMBER : 6906
PROJECT TITLE : Biological Effects of Smoke
PROJECT LEADER : G. J. Patskan
PERIOD COVERED : February, 1991

I. JB6 MOUSE EPIDERMAL CELL TRANSFORMATION ASSAY (G. Nixon)

A. **Objective:** To obtain a colony formation response to 2R1 whole smoke treatment or 2R1 gas phase in soft agar.

B. **Results:** The soft agar assay testing the effects of 0.5 to 2 puffs of 2R1 whole smoke was completed. In this experiment, there was some toxicity at the 2-puff level, an effect not seen to the same extent in an earlier experiment which involved agar exposed to 4 puffs and subsequently diluted down to the 2-puff level. The original 4-puff experiment has been repeated. Two experiments testing the effects of 1-4 puffs of 2R1 gas phase (Cambridge filter assembly inserted into the smoking machine flow) were begun. Results of the first experiment did not show colony formation above control levels.

C. **Plans:** Obtain colony count data for the second 4-puff whole smoke and gas phase experiments.

D. **References:**

Burruss, T. J. Notebook No. 8896, p. 36.

Nixon, G. M. Notebook No. 8711, pp. 167-168.

Vaughan, B. G. Notebook No. 8948, p. 111.

II. SALMONELLA/MICROSOME (S/M) ASSAY (D. Stagg)

A. **Objective:** To test the biological activity of experimental CSCs and other pertinent materials.

B. **Results:** Five CSC's were tested this month in support of the Cross Soluble Base Web studies. The results have been reported in memos.

C. **Plans:** Complete ongoing studies and continue to test samples for biological activity as they become available. Continue to evaluate the "screening" assay protocol as it is currently performed.

D. References:

Jones, R. Notebook No. 8769, p. 108.

Stagg, D. Notebook No. 9038, p. 114.

III. PLANT TISSUE CULTURE (M. Shulleeta)

A. Objective: To develop procedures for the establishment, maintenance and transformation of plant cell cultures.

B. Results: Procedures for the isolation and purification of *N. Tabacum* cv Burley 21 protoplasts have been established. In order to stabilize the protoplast membranes during electroporation and to determine optimal conditions for electroporation, experiments have been conducted to assess the effect of bovine serum albumin (BSA) addition to the medium at varying levels and varying voltage/capacitance settings. Preliminary observations indicate that the number of protoplasts which survive electroporation is higher in BSA treated preparations than in nontreated controls, however, quantitative results are not yet available to confirm these observations or to determine the voltage/capacitance conditions which result in at least 30% survivability.

Axenic cultures of *N. tabacum* cv Kentucky 14 and *N. tabacum* cv Speight G-28 have been initiated as additions to the stock cultures already maintained. Seeds for 2 *N. rustica* cultivars have also been obtained (Brazilia 48 and Brazilia R-120) for axenic culture generation.

C. Plans: Optimize the medium and voltage/capacitance conditions for electroporation of Burley 21 protoplasts.

D. References:

Shulleeta, M. Notebook No. 8961.